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NOTE ON THE INFLUENCE OF SURFACE- EVAPORATION UPON THE DISTRI- BUTION OF INFUSORIA.

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During the course of my experiments on the chemotaxis of *Paramæcium* and *Colpodium*¹ I was struck by the fact that in certain media the infusoria showed a remarkable attraction to the edge of the fluid under the cover-glass — these media were *N*/25 methyl alcohol, *N*/50 CaCl_2 , *n*/5,000 NaOH and *N*/5,000 KOH . This is contrary to the usual behavior of the organisms, in other saline media, in sugar solutions and in the culture medium they avoid the edges of the film of water under the cover-glass and, if undisturbed, form a cluster in the middle of the film, or, if the cover-glass be supported at one end, a little away from the center, towards the supported end.²

Jensen attributed this to the increase in concentration at the edges of the film due to evaporation. Since infusoria appear to be very generally attracted by solutions of lower concentration they tend to congregate in the more dilute part of the film, that is, near the center. In considering the apparent contradiction to this rule displayed by the infusoria when immersed in the above-mentioned media it struck me that in all these cases we had the converse of Jensen's experiment — for these media tend to become more *dilute* at the edge of the film. Thus methyl alcohol evaporates more rapidly than water, so that a solution of methyl alcohol becomes more dilute as it stands exposed to the atmosphere — while solutions of highly hygroscopic substances CaCl_2 , NaOH and KOH absorb water-vapor from the atmosphere and so also tend to become more dilute at the surface. In order to see whether this was the case I tried the effect of adding

¹ *Journal of Biological Chemistry*, January, 1906.

² Jensen, *Pflüger's Archiv. für ges. physiol.*, Vol. 53 (1893), p. 428.

different reagents to the culture-medium, in which the infusoria were suspended, upon their distribution under the cover-glass.

When a substance which evaporates more rapidly than water is dissolved in a watery medium containing salts in solution two contrary effects take place on evaporation — the solution becomes more dilute in respect to the more rapidly evaporating substance owing to its evaporation and more concentrated in respect to the salts owing to the evaporation of water. When the decrease in osmotic pressure due to the evaporation of the more rapidly evaporating substance is greater than the increase in osmotic pressure due to the increased concentration of the salts — then the total result is a decrease in the osmotic pressure. But if the initial concentration of the more rapidly evaporating substance be less than that necessary to bring about the above result then the increase in concentration of the salts will proceed more rapidly than the decrease in concentration of the volatile substance and the total result will be an increase in osmotic pressure at the surface of the fluid. Thus it was to be expected that while under the ordinary conditions of my experiments on *Chemotaxis* — when to a very small amount of culture a comparatively large amount of solution was added — methyl alcohol at a concentration of $N/25$ caused a dispersion of the infusoria to the edge of the cover-glass, when I added 1 c.c. of a solution of methyl alcohol to 5 c.c. of culture it was found necessary to bring the final concentration of the methyl alcohol up to $\frac{5}{6}N$ in order to get the first indication of a tendency to seek the edge — since in this latter case the salts were correspondingly less dilute. The solution of the volatile or of the water-absorbing substance in the culture medium having been made, drops of the mixture were placed on a slide under a cover-glass which was slightly raised at one end.

The following are the experimental results :

Methyl Alcohol. — 1 c.c. of a $5N$ solution of CH_3OH was added to 5 c.c. of the culture-medium in which numerous colpodia and paramoecia were suspended. Result at first uniform distribution of the infusoria under the cover-glass, in a few minutes, however, they began to congregate at the edges — especially at the edges farthest away from the supported end. In a short time all

parts of the film, except the edges, were free from infusoria, while dense clusters had been formed along the edges farthest from the supported end. When a 6*N* solution of methyl alcohol was used instead of a 5*N* solution the effect was more marked and appeared more quickly.

Ethyl Alcohol. — 1 c.c. of a 5*N* solution of C_2H_5OH was added to 5 c.c. of culture. The same effect was obtained as with methyl alcohol, but it was slightly less marked and took longer to appear.

Propyl Alcohol. — $\frac{5}{6}$ *N* C_3H_7OH was too toxic — the organisms being killed too soon to obtain any result — but in $\frac{3}{10}$ *N*, which is about the strongest solution they will stand, there was no attraction to the edges of the film.

Ethyl Acetate. — 1 c.c. of a saturated aqueous solution of ethyl acetate was added to 5 c.c. culture. The organisms were at first uniformly distributed — they then showed marked attraction for the edges of the film, particularly for those edges most remote from the supported end — and in a few minutes all the infusoria were congregated at the edges at the shallower end of the film.

Calcium Chloride. — 1 c.c. of a $\frac{2}{5}$ *N* solution of $CaCl_2$ was added to 5 c.c. of the culture — attraction to the edges was observable for a short time. As in the other cases the edges farthest from the supported end became the most densely populated.

Potassium and Sodium Hydroxides. — In my experiments on *Chemotaxis* and under the conditions of those experiments I obtained a marked, though more or less transient, attraction to the edges of the film at a concentration of $N/5,000$.

All the above results hold equally for *Colpodium* and for *Paramecium*.

In all cases the attraction to the edges finally disappeared — with ethyl acetate the effect passes off in about half an hour.

All these substances which I have found to cause aggregation of the infusoria at the edges of the film are substances the solutions of which tend to become more dilute on exposure to the atmosphere. Methyl alcohol, ethyl alcohol, and ethyl acetate evaporate more rapidly than water while $CaCl_2$, KOH and NaOH are well known absorbents of water-vapor. There is also a general correspondence between the rate at which the volatile substances evaporate and the intensity of the effect which they pro-

duce — thus methyl alcohol has a lower boiling-point than ethyl alcohol and it also causes a more marked attraction to the edges of the film.

The fact that in all these cases the attraction to the edges of the film disappears after a certain time may perhaps be due to the volatile substance having become so dilute in the film that the increase in concentration of the salts due to the evaporation of water now takes place more rapidly than the decrease in concentration of the volatile substance. While in the case of water-absorbing substances — they may have become so dilute that the water-absorption due to their presence in the solution now goes on less rapidly than the loss of water due to evaporation. It seems probable therefore that these phenomena of attraction to or repulsion from the edges of the film are in reality special cases of osmotaxis.

I have alluded to the fact, mentioned by Jensen in the paper to which I have referred, that under ordinary circumstances *Paramæcia* under a cover-glass supported at one end tend to collect in the middle of the film but towards the supported end. But when the substances which cause the infusoria to congregate at the edges of the film are added to the culture we obtain precisely the converse effect — the infusoria collect at the edges, *especially at the edges farthest from the supported end*. It thus appears probable that this is also an osmotactic phenomenon connected with surface dilution. One would be inclined to fancy that it was due to surface evaporation having more effect upon the concentration of the small bulk of liquid at the shallower end of the film than upon the greater bulk of liquid at the supported end — but the *ratio* of surface to volume is not greater at the shallower end than at the supported end. Dr. Loeb has suggested to me that it may be due to the fact that diffusion and therefore equalization of concentration is less rapid in capillary spaces than in the bulk of the liquid — that, in fact, the shallow end of the film may act in a manner analogous to Liebreich's "dead space."¹

¹ *Zeitschrift für Physikalische Chemie*, Vol. V. (1890), p. 529 and Vol. VIII. (1891), p. 83.

CONCLUSIONS.

1. That whereas, under ordinary circumstances, infusoria tend to collect near the center of a film under a cover-glass, when methyl alcohol, ethyl alcohol, ethyl acetate, CaCl_2 , KOH or NaOH are added in sufficient concentration to the culture-medium, this tendency is reversed and the organisms now gather at the edges of the film.

2. That this lends support to Jensen's explanation of the normal tendency of the infusoria to gather near the center of the film, namely, that it is a special case of osmotaxis brought about by surface-evaporation.